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Linezolid against penicillin-sensitive and -resistant pneumococci in the rabbit meningitis model

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Linezolid, a new oxazolidinone antibiotic, showed good penetration (38 ± 4%) into the meninges of rabbits with levels in the CSF ranging from 9.5 to 1.8 mg/L after two iv injections (20 mg/kg). Linezolid was clearly less effective than ceftriaxone against a penicillin-sensitive pneumococcal strain. Against a penicillin-resistant strain, linezolid had slightly inferior killing rates compared with the standard regimen (ceftriaxone combined with vancomycin). *In vitro*, linezolid was marginally bactericidal at concentrations above the MIC (5 × and 10 × MIC).

Introduction

Since the 1970s, the continuous worldwide spread of penicillin-resistant pneumococci has complicated the treatment of pneumococcal infections.[1] However, β-lactams have remained the first line drugs, provided that their penetration into infected tissues is sufficient (e.g. into the lung).[2] In meningitis, because of the limited penetration into the subarachnoid space, the therapeutical options are more restricted. A combination of cephalosporins (i.e. ceftriaxone or cefotaxime) is usually recommended as an empirical regimen.[3,4] The problem of penicillin-resistant strains is complicated by the associated resistance to cephalosporins. Moreover, there have been several reports of failures following treatment with ceftriaxone or cefotaxime in pneumococcal meningitis caused by cefotaxime-resistant strains.[5,6] Even clinical failures with vancomycin have been reported, which may be a result of the variable penetration of vancomycin into the CSF, particularly when it is administered in association with dexamethasone.[7] This underlines the necessity of alternative therapies for pneumococcal meningitis caused by resistant strains.

Among the recently developed drugs, oxazolidinone antibiotics are promising candidates. This new class of antibacterial agents acts by inhibiting protein synthesis.[8,9] These substances are active against Gram-positive microorganisms, including penicillin-sensitive and -resistant pneumococci.[10,11] The aim of the present work was to study the penetration of linezolid, a new fluorinated oxazolidinone derivative, into inflamed meninges and to test its efficacy against penicillin-sensitive and -resistant pneumococci in experimental meningitis and *in vitro*. Ceftriaxone monotherapy was used as standard regimen against a penicillin-sensitive isolate and ceftriaxone combined with vancomycin as a standard regimen against a penicillin-resistant strain.

Materials and methods

Pneumococcal strains

A penicillin-sensitive and a penicillin-resistant pneumococcal strain were used in experiments *in vitro* and in the meningitis model. Both strains were originally isolated from patients with pneumonia at the University Hospital of Berne (Switzerland). The MICs against the penicillin-resistant strain serogroup 6 were the following: penicillin G 4 mg/L; vancomycin 0.12–0.25 mg/L; ceftriaxone 0.5 mg/L; and linezolid 0.5 mg/L. The MICs against the penicillin-sensitive strain were the following: penicillin G 0.06 mg/L; ceftriaxone 0.05 mg/L; and linezolid 0.5 mg/L. MICs were determined in liquid cultures.

Rabbit meningitis model

The meningitis model originally described by Dacey & Sande[12] was used in a slightly modified way. The experimental protocol was approved by the Kantonales Amt für

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Veterinärmedizin, Berne, Switzerland. In brief, young New Zealand white rabbits weighing 2–2.5 kg (provided by the Zentraltierställe der Medizinischen Fakultät, Berne) were anaesthetized by im injections of ketamine (30 mg/kg) and xylazine (15 mg/kg), and were immobilized in stereotactic frames for induction of meningitis and CSF sampling. An inoculum containing 1×10^5 cfu of either the penicillin-sensitive strain or the penicillin-resistant pneumococcus was injected directly into the cisterna magna. A long-acting anaesthetic (ethylcarbamate or urethane, 3.5 g/rabbit) was injected subcutaneously. The animals were then returned to their cages. Fourteen hours later, the cisterna magna was punctured again for periodic CSF sampling at 0, 1, 2, 4, 5, 6 and 8 h, and a catheter was introduced in the femoral artery for serum sampling at 0, 0.25, 0.5, 1, 2, 4, 4.25, 4.5, 5, 6 and 8 h. Antibiotics were administered through a peripheral ear vein as bolus injections at the following concentrations: ceftriaxone 125 mg/kg; vancomycin 20 mg/kg; linezolid 20 mg/kg. Ceftriaxone was injected at 0 h. Vancomycin and linezolid were administered at 0 and 4 h. Antibiotics were purchased commercially (vancomycin, ceftriaxone) and linezolid was kindly provided by Pharmacia and Upjohn (Kalamazoo, MI, USA). Untreated controls received the same volume of saline. At the end of the experiment all animals received a lethal dose of nembutal.

Bacterial viability was measured by 10-fold serial dilutions of all CSF samples, plated on blood agar plates containing 5% sheep blood and incubated overnight at 37°C. The efficacy of the antimicrobial treatment was determined by decrease of viable cell count over 8 h. In addition, the antimicrobial activity of the regimens was calculated by linear regression analysis and expressed as decrease of $\log_{10}$ cfu/mL/h ($\Delta\log_{10}$ cfu/mL/h). In parallel, 20 μL of undiluted CSF was plated (= limit of detectability: 50 cfu/mL). The different dilutions were compared in order to exclude significant carryover effects during therapy. We arbitrarily assigned a value of 1.7 (= $\log_{10}$ of the limit of detectability) to the first sterile CSF sample and a value of 0 to any following sterile sample. The results are expressed as mean ± S.D. Statistical significance was determined by the Newman–Keuls and the Tukey multiple comparison tests.

Measurement of antibiotic levels in serum and CSF

Linezolid concentrations in serum and CSF were determined by high-performance liquid chromatography (HPLC) (Drs M.-S. Kuo and R. Zielinsky, Pharmacia and Upjohn). The CSF penetration of linezolid was calculated by comparison of serum and CSF areas under the curve (AUC) for all animals (Systat software, SSPS Inc., Evanston, IL, USA).

In vitro assays

The pneumococcal strains (penicillin-sensitive and -resistant) were grown in C+Y medium[13] to optical density 0.3 at 590 nm and then diluted 40-fold to 10^6 cfu/mL, corresponding to the CSF bacterial titre in rabbits before initiation of therapy. Linezolid was added in concentrations ranging from 0.5 to 5 mg/L corresponding to 1 ×, 5 × and 10 × MIC. Bacterial titres were determined at 0, 2, 4, 6 and 8 h by serial dilution of samples, plated on agar plates containing 5% sheep blood and incubated at 37°C for 24 h. Experiments were performed in triplicate and results expressed as mean ± S.D.

Results

Kinetic data of linezolid are presented in Figure 1. Linezolid (20 mg/kg) produced a peak serum level of approximately 69 mg/L, decreasing to 2.5 mg/L 4 h later. The second injection led to a peak level around 66 mg/L and a trough level of 4 mg/L. CSF levels peaked at 9.5 mg/L after the first injection, decreasing slowly to 1.8 mg/L 4 h later. The second injection produced a peak level around 10.5 mg/L and a trough level of 2 mg/L at the end of the experimental period. In our rabbit model, the penetration of linezolid into inflamed meninges was 38 ± 4%. The CSF linezolid levels remained above the MIC (0.5 mg/L) during the entire treatment period. The CSF:MIC ratio ranged from 19 to 3.6 during the first 4 h and from 21 to 4 during the last 4 h.

The antibacterial activity of linezolid against penicillin-sensitive and -resistant strains in time-course viable count experiments over 8 h *in vitro* is presented in Figures 2 and 3. *In vitro*, linezolid had a comparable antibacterial activity against penicillin-sensitive and -resistant strains ($P > 0.05$). Concentrations corresponding to 1 ×, 5 × and 10 × the MIC produced only a minimal bactericidal activity (1.3 and

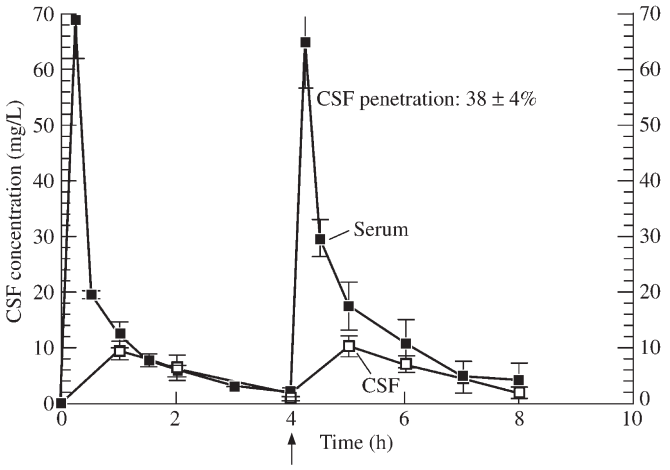

Figure 1. Linezolid concentrations in serum and CSF for 8 h after iv injections of 2 × 20 mg/kg linezolid. The arrow represents the time point of the second linezolid injection. The concentration of linezolid remained greater than the MIC (0.5 mg/L) during the entire period of therapy.

1 $\log_{10}$ cfu/mL for 10 × MIC against the penicillin-sensitive and -resistant strain, respectively).

The killing rates of the different substances are summarized in the Table. Before therapy the bacterial titre was comparable in all groups. Against penicillin-sensitive isolates, ceftriaxone was clearly superior to linezolid ($P < 0.05$). The standard regimen (ceftriaxone combined with vancomycin) was significantly ($P < 0.05$) more effective than linezolid or ceftriaxone monotherapy against penicillin-resistant strains. It is interesting to note that linezolid tended to have a more pronounced antibacterial activity against the penicillin-resistant strain than against the penicillin-sensitive pneumococci.

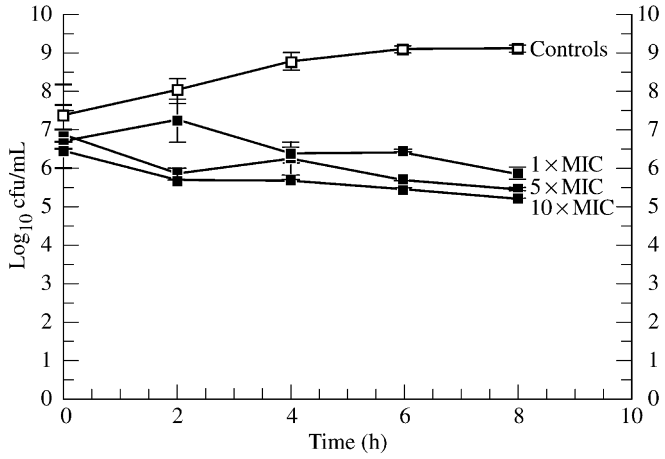

Figure 2. Killing rates of linezolid with concentrations ranging from 0.5 mg/L (1 × MIC) to 5 mg/L (10 × MIC) against a penicillin-sensitive pneumococcal isolate. Open squares represent untreated controls. Experiments were performed in triplicate and killing rates are expressed as mean ± S.D.

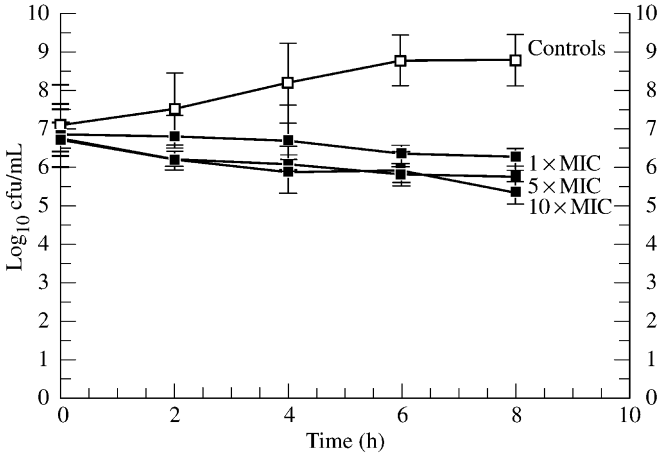

Figure 3. Killing rates of linezolid with concentrations ranging from 0.5 mg/L (1 × MIC) to 5 mg/L (10 × MIC) against a penicillin-resistant pneumococcal strain. Open squares represent untreated controls. Experiments were performed in triplicate and killing rates are expressed as mean ± S.D.

Table. Linezolid in experimental meningitis caused by *Streptococcus pneumoniae* sensitive (Pen-S) and resistant (Pen-R) to penicillin

Antibiotic	No. of rabbits	Initial titre [$\log_{10}$ cfu/mL (mean ± S.D.)]	Killing rate [$\Delta \log_{10}$ cfu/mL/h (mean ± S.D.)]	Killing rate/8 h [$\log_{10}$ cfu/mL (mean ± S.D.)]
Controls (Pen-S)	8	6.37 ± 0.71	+0.06 ± 0.10	+0.26 ± 0.12[a]
Linezolid (Pen-S)	10	6.05 ± 1.43	–0.25 ± 0.08	–1.33 ± 0.69[b]
Ceftriaxone (Pen-S)	5	5.64 ± 0.86	–0.90 ± 0.27	–5.53 ± 0.70[b]
Controls (Pen-R)	6	6.32 ± 0.52	+0.10 ± 0.06	+0.39 ± 0.10[a]
Linezolid (Pen-R)	16	5.95 ± 1.40	–0.41 ± 0.20	–2.50 ± 1.14[c]
Ceftriaxone (Pen-R)	7	6.31 ± 0.20	–0.31 ± 0.20	–1.90 ± 0.53[c]
Ceftriaxone + vancomycin (Pen-R)	7	6.33 ± 0.25	–0.55 ± 0.19	–3.90 ± 0.65[c]

[a] $P < 0.05$ versus all groups, by Newman–Keuls multiple comparison test.
[b] $P < 0.05$ ceftriaxone versus linezolid.
[c] $P < 0.5$ ceftriaxone + vancomycin versus linezolid or ceftriaxone monotherapy, by Tukey multiple comparison test.

Discussion

Before the emergence of penicillin- and cephalosporin-resistant strains, β-lactam antibiotics, especially cephalosporins, were the first line antibiotics for treatment of pneumococcal meningitis.[4] Recently documented failures of treatment with cephalosporin monotherapies[5,6] underline the need for alternative therapeutical regimens. Based on their good activity *in vitro* against penicillin-sensitive and -resistant pneumococci, oxazolidinone antibiotics might be useful in the treatment of pneumococcal meningitis.

Little is known about the effectiveness of linezolid in pneumococcal meningitis.

In the rabbit meningitis model, we compared linezolid with the standard regimens: ceftriaxone against a penicillin-sensitive pneumococcal strain and ceftriaxone combined with vancomycin against a penicillin-resistant isolate.

Since the dosage of linezolid for the treatment of meningitis has not been clearly defined, we deliberately chose a dosage (2×20 mg/kg) higher than levels achieved in humans after one injection of 375 or 625 mg linezolid. Linezolid (20 mg/kg) produced peak levels ranging from 69 to 64 mg/L in rabbits, whereas 375 mg injected intravenously produces peak levels around 11 mg/L in humans. The trough levels (4–2.5 mg/L) in rabbits were reached after 4 h and corresponded to levels measured in humans after 8 h (2.5 mg/L).[14] This provided the rationale for administering a second dose of linezolid after 4 h. The dosages of vancomycin (2×20 mg/kg) and ceftriaxone (1×125 mg/kg) used in our study were standard dosages used in previous studies with the same experimental model.[15]

With this dosing regimen, linezolid CSF levels remained above the MIC during the entire treatment period with CSF:MIC ratios ranging from 21 to 3.6.

In light of the good penetration ($38 \pm 4\%$) of linezolid into the CSF, the poor antibacterial activity of linezolid compared with the standard regimen (ceftriaxone) against penicillin-sensitive strains is unclear. The lack of efficacy of linezolid can be explained by the marginal bactericidal activity of linezolid documented *in vitro*, since a highly bactericidal activity is one of the major prerequisites for the treatment of pneumococcal meningitis.

It is interesting to note that linezolid seemed to show a slightly more pronounced activity against a penicillin-resistant strain, but based on our data *in vitro* (Figures 2 and 3), the reasons for this enhanced efficacy are unclear. On the other hand, the killing rates of linezolid were inferior to the standard regimen (ceftriaxone combined with vancomycin).

In summary, the place of linezolid in the treatment of pneumococcal meningitis is uncertain. On one hand, based on our experimental data, we cannot recommend the use of linezolid against penicillin-sensitive strains, but on the other hand, linezolid could be a conceivable alternative against penicillin-resistant pneumococci. The efficacy of linezolid in the treatment of pneumococcal diseases caused by penicillin-resistant strains deserves further investigation.

Acknowledgement

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